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(57) Abstract

Broad spectrum hydroxamic acid and carboxylic acid derivatives, previously known in the art as inhibitors of matrix metalloproteinases such as collagenase are capable of inhibiting the production and processing of transforming growth factor alpha (TGF-α) by cells, and thus are useful in the management of diseases or conditions mediated by overproduction of, or over-responsiveness to, TGF-α.

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# USE OF MATRIX METALLOPROTEINASE INHIBITORS FOR THE TREATMENT OF DISEASES MEDIATED BY TNF-ALPHA

## Field of the Invention.

This present invention relates to the novel use of certain hydroxamic acid and carboxylic acid derivatives, previously known in the art as inhibitors of matrix metalloproteinases such as collagenase, for inhibiting the production and processing of transforming growth factor alpha (TGF-α) by cells, and thus is useful in the management of diseases or conditions mediated by overproduction of, or over-responsiveness to, TGF-α.

## Background to the invention.

Transforming growth factor alpha (TGF-α) is a potent mitogen for fibroblasts, keratinocytes, erythroid progenitor cells and many tumours, which has 35% homology and similar biological activity to epidermal growth factor (EGF). In common with EGF, TGF-α binds with high affinity to the 170kDa EGF receptor resulting in autophosphorylation of the receptor, activation of the tyrosine kinase and initiation of the cascade of intracellular signalling events leading to cell division (R Brachmann *et al.* (1989) Cell. **56(4)**:691-700). In addition to its mitogenic activity, TGF-α is known to stimulate epithelial cell migration, promote angiogenesis, induce bone resorption, stimulate hypercalcemia and inhibit gastric acid secretion (R Derynck (1992) Advances in Cancer Research. **58**:27-52; R Derynck (1988) Cell. **54**:593-595; Schreiber *et al.* (1986) Science. **232**:1250-1253). TGF-α, which is also constitutively produced by human blood eosinophils, may participate in the inflammatory reaction by interacting with mesenchymal and epithelial cells, thus promoting fibrosis or neovascularization (Xalz *et al.* (1993) Leukemia. **7(10)**:1531-1537).

TGF-α is expressed in embryonic tissues and also in a wide range of normal cells and tissues including: keratinocytes, macrophages, eosinophils, mammary epithelia, corneal epithelia, gastric mucosa, pancreas, pituitary and brain. Overexpression of

TGF-α promotes transformation and anchorage-independent growth of cells *in vitro*, and enhanced production of TGF-α is frequently observed in neoplasia (Massagué (1990) J. Biol. Chem. **35**:21393-21396; N Normanno *et al.* (1994) Breast Cancer Research and Treatment. **29**:11-27; DT Wong (1993) Oral Oncology. European J. Cancer Part B, **29B**:3-7). It is believed that this overexpression of TGF-α, coupled with the simultaneous overexpression of the EGF receptor may result in overstimulation of the autocrine and paracrine signalling pathways, resulting in abnormal cell growth and the generation or progression of neoplasia. This is supported by the high recorded incidence of mammary and liver neoplasias in transgenic mice that chronically overexpress TGF-α (Y Matsui *et al.*(1990) Cell. **61**:1147-1155).

The TGF-α precursor is a 160 amino acid, 20-22 kDa polypeptide with a hydrophobic transmembrane sequence of 23 amino acids and is oriented with the N-terminus on the extracellular side of the membrane. The soluble 50 amino acid form of TGF-α is generated by cleavage of pro-TGF-α between residues Ala38-Val39 and Ala88-Val89. Other possible cleavage sites exist in the extracellular domain including a Lys95-Lys96 close to the cell membrane spanning region. Newly synthesised pro-TGF-α appears to be resistant to cleavage until it reaches the plasma membrane, where in CHO cells transfected with a gene encoding the precursor, cleavage is known to occur in at least two stages. In the first stage, pro-TGF-α is cleaved at the site proximal to the N- terminus, thereby shortening the membrane-anchored precursor to 17kDa. This cleavage step appears to proceed rapidly with a $t_{1/2}$ of 15 minutes. The second step, which is rate limiting, involves cleavage at the second site distal to the N-terminus, releasing the soluble, bioactive form into the medium along with the 5kDa glycosylated N-terminus. This terminal portion of the molecule has no known function. In non-stimulated cells this second stage of the cleavage process has a $t_{1/2}$ of about 4 hours, leading to the accumulation of pro-TGF-α on the cell surface. Stimulation of secretion via protein kinase C-dependent or independent mechanisms dramatically decreases the $t_{1/2}$ of this second step to about 5 minutes resulting in a very rapid turnover of the cell surface precursors. Some tumour-derived

or retrovirally transformed cells release soluble forms that contain the N-terminal glycosylated region of the precursor still linked to the mature 6kDa TGF-α sequence, suggesting that regulation of cleavage differs between different cell types. These forms are referred to as meso-TGF-α. It is not known if the meso forms of TGF-α differ in function to the final 50 amino acid product. The enzyme or enzymes responsible for the cleavage of pro-TGF-α to the mature form have not been identified.

Neovascularisation is a critical component for normal cell growth, for injury repair and particularly for the development of the vascular system. There are however, clinical hyperplastic conditions characterised by abnormal neovascularisation, for example psoriasis and diabetic retinopathy. Abnormal neovascularisation is also associated with neoplastic cancer conditions. The development of solid tumors and unrestricted tumour growth necessitates the induction of angiogenesis to provide a network of blood capillaries in order to provide nourishment for the tumour cells, and to remove waste metabolites from the tumour cells.

The demonstration that inhibition of angiogenesis is associated with tumour regression has provided the most compelling evidence for the role of angiogenesis in tumour growth, and it is therefore generally recognised that agents capable of inhibiting tumour cell mediated neovascularisation will prove useful in inhibiting the growth of tumours. Moreover, agents capable of inhibiting or regressing capillary formation in general may also be therapeutically useful in treating all diseases which are accompanied by angiogenesis.

It is therefore desirable to identify factors that inhibit angiogenesis. Accordingly, WO 94/18967 discloses the use of a particular class of imidazoles useful in inhibiting angiogenesis, EP application 424,193 discloses the use of actinonin as an inhibitor of angiogenesis, and WO 93/16716 teaches that various peptide fragments of thrombospondin are capable of inhibiting angiogenesis. Inhibitors of matrix metalloproteinases have also been implicated in inhibiting angiogenesis (Tamargo *et al.* (1991) Cancer Res. **51**:672-675; Fischer *et al.* (1994) Dev. Biol. **162**:499-510 and Galardy *et al.* (1994) Cancer Res. **54**:4715-4718).

There is considerable evidence that TGF-α is a critical mediator in the pathology of several diseases. TGF-α is elevated in skin lesions of psoriasis and of ultraviolet-induced skin hyperplasia, in wounds leading to scar and keloid formation, and in breast cancer, liver cancer, squamous carcinoma, ovarian carcinoma and keratoacanthoma. Transgenic mice overexpressing TGF-α display hyperproliferative skin lesions similar to psoriasis, and are prone to develop liver and mammary tumours. Henry *et al.* (1987; Proceedings,78th Ann. meeting Amer. Soc. Cancer Res. **28**:60 abstract 238) reported that three human breast cancer cell lines produce substantial amounts of TGF-α compared to normal cells, and therefore that growth of human breast cancer cells may be regulated in part by autocrine synthesis of TGF-α. Blocking the action of TGF-α is anti-proliferative for several tumour cell lines in culture, and can inhibit growth of tumour explants in nude mice.

Blocking production of TGF-α should be beneficial in malignant disorders where TGF-α is an autocrine or paracrine growth factor for the tumour. where TGF-α mediates angiogenesis associated with tumour growth, or where TGF-α mediates bone resorption and hypercalcemia. Blocking production of TGF-α should also be beneficial in conditions of hyperplasia (non-cancerous cell proliferation) such as fibrolytic conditions including skin disorders such as eczema. In inflammatory diseases blocking TGF-α production should be beneficial where TGF-α is acting as a growth factor for connective tissue cells, mediating hyperproliferation, or where excessive neo-vascularisation occurs.

TGF-α stimulates TGF-ß in speeding wound healing, which leads to scar and keloid formation. It would therefore be desirable to be able to provide a therapeutic agent to inhibit TGF-α, leading to a reduction in the pathological actions of TGF-α.

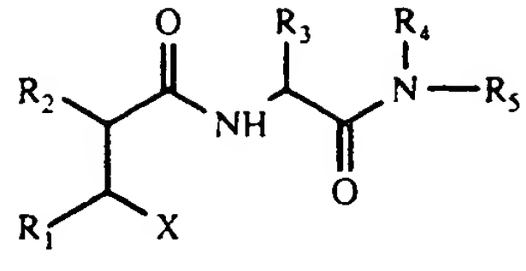
There is one published report which states that processing of TGF-α is not inhibited by matrix metalloproteinase inhibitors (McGeehan GM *et al.* (1994) Nature. **370**:558-

561).

The present invention is based on the finding that broad spectrum matrix metalloproteinase inhibitors capable of inhibiting collagenase, stromelysin and gelatinase are also effective in inhibiting TGF-α release. Many compounds are known which are active as broad spectrum matrix metalloproteinase inhibitors and which are not believed to be cytotoxic, and the use of such compounds may therefore provide a breakthrough in the management of diseases mediated by TGF-α; diseases for example where angiogenesis is important, such as diabetic retinopathy, pterygia, psoriasis and atherosclerosis, or other hyperplastic diseases such as fibrinolytic diseases or ultraviolet-induced skin hyperplasia or in the control of wound healing.

Metalloproteinases are characterised by the presence in the structure of a zinc(II) ionic site. It is now known that there exists a range of metalloproteinase enzymes that includes fibroblast collagenase (Type 1), PMN-collagenase, 72 kDa-gelatinase, 92 kDa-gelatinase, stromelysin, stromelysin-2 and PUMP-1 (J.F. Woessner, FASEB J, 5:2145-2154, 1991). Many known MMP inhibitors are peptide derivatives, based on naturally occurring amino acids, and are analogues of the cleavage site in the collagen molecule. A recent paper by Chapman *et al.* (J. Med. Chem. **36**:4293-4301, 1993) reports some general structure/activity findings in a series of N-carboxyalkyl peptides. Other known MMP inhibitors are less peptidic in structure, and may more properly be viewed as pseudopeptides or peptide mimetics. Such compounds usually having a functional group capable of binding to the zinc (II) site in the MMP, and known classes include those in which the zinc binding group is a hydroxamic acid, carboxylic acid, sulphydril, and oxygenated phosphorus (eg phosphinic acid and phosphonamidate including aminophosphonic acid) groups.

Two known classes of pseudopeptide or peptide mimetic MMP inhibitors have a hydroxamic acid group and a carboxylic group respectively as their zinc binding groups. With a few exceptions, such known MMPs may be represented by the structural formula (I)



(I)

in which X is the zinc binding hydroxamic acid (-CONHOH) or carboxylic acid (-COOH) group and the groups $R_1$ to $R_5$ are variable in accordance with the specific prior art disclosures of such compounds. Examples of patent publications disclosing such structures are given below.

In such compounds, it is generally understood in the art that variation of the zinc binding group and the substituents $R_1$, $R_2$ and $R_3$ can have an appreciable effect on the relative inhibition of the metalloproteinase enzymes. The group X is thought to interact with metalloproteinase enzymes by binding to a zinc(II) ion in the active site. Generally the hydroxamic acid group is preferred over the carboxylic acid group in terms of inhibitory activity against the various metalloproteinase enzymes. However, the carboxylic acid group in combination with other substituents can provide selective inhibition of gelatinase (EP-489,577-A). The $R_1$, $R_2$ and $R_3$ groups are believed to occupy respectively the P1, P1' and P2' amino acid side chain binding sites for the natural enzyme substrate. There is evidence that a larger $R_1$ substituent can enhance activity against stromelysin, and that a ($C_1$-$C_6$)alkyl group (such as iso-butyl) at $R_2$ may be preferred for activity against collagenase whilst an alkylphenyl group (such as phenylpropyl) at $R_2$ may provide selectivity for gelatinase over the other metalloproteinases.

Summary of the invention.

Although matrix metalloproteinase inhibitors (MMPIs) are disclosed for applications where such enzymes especially collagenase, stromelysin and gelatinase are the major causative mechanism, the art, for example WO 93/13741, also contains speculative claims suggesting that such inhibitors might be useful in inflammation and hyperplasia conditions such as psoriasis. The rationale for such claims is that matrix

metalloproteinases cause the breakdown of the connective tissue thus enabling neovascularisation to develop. In contrast however, there is a sound basis for implicating TGF-α as the primary causative factor in the angiogenic and fibrotic disease states and the present invention has shown that broad spectrum MMPIs are capable of preventing the production of TGF-α and therefore justifiably demonstrates their utility in treating hyperplasia conditions. The present invention has therefore opened up these disease end-points as targets treatable by broad spectrum hydroxamic acid- and carboxylic acid MMPIs.

The present invention therefore concerns a method of management of diseases mediated by TGF-α in mammals, in particular humans, the method comprising administering to the mammal an effective amount of a non-endogenous matrix metalloproteinase inhibitor or a pharmaceutically or veterinary acceptable salt thereof.

Matrix metalloproteinases have been shown to play an important role in the cancer-mediated degradation of extracellular matrix. This matrix acts as a "host-defence" mechanism in xenograft models of cancer spread, forming an impenetrable barrier (DeVore *et al.*, (1980) Expl. Cell Biol. **48**:367-373). Degradation enables the cancer cells to move through the extracellular matrix, and both to enter and exit the vasculature; these steps are integral to the process of metastasis, which may occur early or late in the growth of the primary tumour, depending on tumour type.

However, the present invention does not relate to the use of MMPIs to inhibit the cancer-mediated degradation of extracellular matrix, or in metastasis, but rather to the novel use of certain broad spectrum MMPIs in inhibiting TGF-α. This invention therefore extends considerably the known clinical utility of matrix metalloproteinase inhibitors by enabling the treatment of patients with diseases mediated by TGF-α.

Description of the Invention

In a first aspect of the invention, there is provided the use of an exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined herein, in the preparation of an agent for the treatment of conditions or diseases mediated or primarily mediated by transforming growth factor alpha (TGF-α).

As used throughout this specification, a "broad spectrum matrix metalloproteinase inhibitor" (MMPI) means one that exhibits biological activity $IC_{50}$ (50% inhibitory concentration) values of 300nM or less for each of collagenase determined by the procedure of Cawston and Barrett, (Anal. Biochem. **99**:340-345. 1979), stromelysin determined by the procedure of Cawston *et al.* (Biochem. J. **195**:159-165, 1981) and gelatinase determined by the procedure of Sellers *et al.*, (Biochem. J. **171**:493-496,1979).

Another aspect of this invention concerns:

(i) a method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by or primarily mediated by TGF-α in mammals, particularly in humans, which method comprises administration to the mammal an effective dose of a broad spectrum MMPI compound as defined herein, or a pharmaceutically acceptable salt thereof; and

(ii) an exogenous broad spectrum MMPI compound as defined herein, for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by or primarily mediated by TGF-α ; and

(iii) the use of a broad spectrum MMPI compound as defined herein, in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by or primarily mediated by TGF-α.

Salts of the compounds of the invention include physiologically acceptable acid addition salts for example hydrochlorides, hydrobromides, sulphates, methane sulphonates, p-toluenesulphonates, phosphates, acetates, citrates, succinates, lactates, tartrates, fumarates and maleates. Salts may also be formed with bases, for example sodium, potassium, magnesium, and calcium salts.

Diseases or conditions mediated by TGF-α include but are not limited to inflammation, autoimmune disease, wound healing including scar and keloid formation, fibrinolytic disorders, angiogenic disorders and cancer.

Preventing the production or action of TGF-α is predicted to be a potent therapeutic strategy for many disease states. These include, but are not restricted to, psoriasis, scleroderma, hemangioma, diabetic retinopathy, neovascular glaucoma, retrolental fibroplasia, atherosclerosis, arteriovenous malformations, vascular adhesions, arthritis, wound healing , fibrotic disorders, systemic sclerosis, systemic lupus erythematosus, vasculaities, vasculitides, hypercalcemia of malignancy, ovarian carcinoma, breast cancer, liver cancer, squamous carcinoma, ovarian carcinoma, keratoacanthoma, pancreatic carcinoma, colon carcinoma, erythroleukaemia and in any disease state where TGF-α is a mediator of host injury or where TGF-α production is an important feature of the disease pathology.

According to a further aspect of the invention there is provided the use of a broad spectrum matrix metalloproteinase inhibitor compound in the treatment of any of the following diseases or conditions mediated by overproduction of, or over-responsiveness to TGF-α: psoriasis, scleroderma, hemangioma, diabetic retinopathy, neovascular glaucoma, retrolental fibroplasia, atherosclerosis, arteriovenous malformations, vascular adhesions, arthritis, wound healing , fibrotic disorders, systemic sclerosis, systemic lupus erythematosus, vasculaities, vasculitides, hypercalcemia of malignancy, ovarian carcinoma, breast cancer, liver cancer, squamous carcinoma, ovarian carcinoma, keratoacanthoma, pancreatic carcinoma, colon carcinoma or erythroleukaemia.

As mentioned above, MMP inhibitors have been proposed with hydroxamic acid or carboxylic acid zinc binding groups. The following patent publications, which are incorporated herein by reference, disclose hydroxamic acid-based MMP inhibitors:

| | |
|---|---|
| US 4599361 | (Searle) |
| EP-A-2321081 | (ICI) |
| EP-A-0236872 | (Roche) |
| EP-A-0274453 | (Bellon) |
| WO 90/05716 | (British Bio-technology) |
| WO 90/05719 | (British Bio-technology) |
| WO 91/02716 | (British Bio-technology) |
| WO 92/09563 | (Glycomed) |
| US 5183900 | (Glycomed) |
| US 5270326 | (Glycomed) |
| WO 92/17460 | (SmithKline Beecham) |
| EP-A-0489577 | (Celltech) |
| EP-A-0489579 | (Celltech) |
| EP-A-0497192 | (Roche) |
| US 5256657 | (Sterling Winthrop) |
| WO 92/13831 | (British Bio-technology) |
| WO 92/22523 | (Research Corporation Technologies) |
| WO 93/09090 | (Yamanouchi) |
| WO 93/09097 | (Sankyo) |
| WO 93/20047 | (British Bio-technology) |
| WO 93/24449 | (Celltech) |
| WO 93/24475 | (Celltech) |
| EP-A-0574758 | (Roche) |
| EP-A-0575844 | (Roche) |
| WO 94/02446 | (British Bio-technology) |
| WO 94/02447 | (British Bio-technology) |

The following patent publications, which are incorporated herein by reference, disclose carboxylic acid-based MMP inhibitors:

EP-A-0489577 (Celltech)
EP-A-0489579 (Celltech)
WO 93/24449 (Celltech)
WO 93/24475 (Celltech)

The reader is referred thereto for details of the structures of the compounds disclosed and methods for their preparation.

Broad spectrum MMPI compounds may be identified amongst those disclosed in the above publications, by assaying such known MMPIs for their ability to inhibit each of collagenase, stromelysin and gelatinase. Inhibition of these enzymes is assessed using the assays described *infra*.

The potency of compounds as inhibitors of collagenase is determined by the procedure of Cawston and Barrett, (Anal. Biochem. **99**:340-345, 1979), hereby incorporated by reference, or the following adaptation, whereby a 1mM solution of the compound being tested, or a dilution thereof, is incubated at 37°C for 16 hours with collagen and collagenase (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% $NaN_3$). The collagen is acetylated $^{14}C$ collagen prepared by the method of Cawston and Murphy, (Methods in Enzymology. **80**:711, 1981), hereby incorporated by reference. The samples are centrifuged to sediment undigested collagen, and an aliquot of the radioactive supernatant is removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM of the test compound, or a dilution thereof, is compared to activity in a control devoid of inhibitor and the inhibitor concentration effecting 50% inhibition of the collagenase activity ($IC_{50}$) is obtained.

The potency of compounds as inhibitors of stromelysin is determined by the procedure of Cawston *et al.* (Biochem. J. **195**:159-165, 1981), hereby incorporated by reference, or the following adaptation, whereby a 1mM solution of the compound being tested, or a dilution thereof, is incubated at 37°C for 16 hours with stromelysin and $^{14}C$ acetylate casein (buffered with 25mM Hepes, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% $NaN_3$). The casein is acetylated $^{14}C$ casein prepared by the

method of Cawston *et al. (ibid)*. The stromelysin activity in the presence of 1mM of the test compound, or a dilution thereof, is compared to activity in a control devoid of inhibitor and the result of inhibitor concentration effecting 50% inhibition of the stromelysin activity ($IC_{50}$) is obtained.

The potency of compounds as inhibitors of gelatinase is determined by the procedure of Sellers *et al.*, (Biochem. J.**171**:493-496.1979), hereby incorporated by reference, or the following adaptation, whereby a 1mM solution of the compound being tested, or a dilution thereof, is incubated at 37°C for 16 hours with 50ng $^{14}C$ gelatin in 8mM acetic acid, and gelatinase (buffered with 100mM Tris, pH 7.5 containing 5mM $CaCl_2$, 0.05% Brij 35 and 0.02% $NaN_3$). The $^{14}C$ gelatin is freshly prepared by the thermal denaturation (57°C for 10 minutes) of $^{14}C$-labelled collagen. The collagen is acetylated $^{14}C$ collagen prepared by the method of Cawston and Murphy, (Methods in Enzymology. **80**:711, 1981), hereby incorporated by reference. At the end of incubation, 10µl of 0.5% bovine serum albumin and 80µl of 60% TCA (trichloroacetic acid) are added and the samples placed on ice for 15 minutes. The samples are centrifuged to sediment undigested substrate, and an aliquot of the radioactive supernatant is removed for assay on a scintillation counter as a measure of hydrolysis The gelatinase activity in the presence of 1mM of the test compound, or a dilution thereof, is compared to activity in a control devoid of inhibitor and the inhibitor concentration effecting 50% inhibition of the gelatinase activity ($IC_{50}$) is obtained.

$IC_{50}$ values for each of collagenase, stromelysin and gelatinase of 300nM or less identifies broad spectrum MMPI compounds useful in the invention. Any of these broad spectrum MMPI compounds can be used in the treatment of diseases mediated by TGF-α production. but preferred compounds include those which contain a hydroxamic acid or carboxylic acid moiety including *inter alia* : 3R-(1S-methylcarbamoyl-2-phenylethylcarbamoyl)-5-methyl-2S-(thien-2-ylsulphanylmethyl)-hexanohydroxamic acid. 3R-(3-tert-butoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-(4-hydroxy-phenylsulphanylmethyl)-5-methyl-hexanohydroxamic acid. 3R-(2,2-dimethyl-1S-(thiazol-2-ylcarbamoyl)-propylcarbamoyl)-5-methyl-2S-prop-2-enylhexanohydroxamic acid. 2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-

propylcarbamoyl)-5-methyl-hexanohydroxamic acid, 3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-prop-2-enyl-hexanohydroxamic acid or 3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-hydroxy-5-methyl-hexanohydroxamic acid.

In a further aspect of the invention there is provided a pharmaceutical or veterinary composition adapted for inhibition of TGF-α release which composition comprises at least one exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore, in admixture with at least one pharmaceutically or veterinary acceptable carrier.

Suitable MMP inhibitors may be prepared for administration by any route. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practise. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

The active ingredient may also be administered parenterally (e.g. subcutaneous, intramuscular and intravenous) in a sterile medium, which is preferably isotonic with the blood of the recipient. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution.

The dosage unit involved in oral administration may contain from about 1 to 250mg, preferably from about 25 to 250mg of a compound of general formula I *supra*. A suitable daily dose for a mammal may vary widely depending on the condition of the patient. However, a dose of a compound of general formula I of about 0.1 to 300mg/kg body weight, particularly from about 1 to 100mg/kg body weight may be appropriate.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage for topical administration will of course depend on the size of the area being treated. For the eyes, each dose may typically be in the range from 10 to 100mg of the drug.

The following examples illustrate the invention, but are not intended to limit the scope in any way.

**Example 1**

Compound 1 (2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid), a broad spectrum matrix metalloproteinase inhibitor, can prevent the release of TGF-α from cells.

The ability of a representative broad spectrum matrix metalloproteinase inhibitor compound, Compound 1 (2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid), which is a broad spectrum MMPI as defined herein, to inhibit the release of TGF-α was investigated. The assay is based on the ability of broad spectrum matrix metalloproteinase inhibitor compounds to inhibit the action of TGF-α convertase enzyme.

Cells are seeded three days prior to experiment in 1ml/well of a 24-well plate at a density of 2-5x$10^5$/ml of appropriate growth medium, in order to generate a confluent monolayer of cells on test day.

The confluent monolayers of the test cell-line are washed to remove all traces of foetal calf serum. Cells are then allowed to condition serum free culture medium (Assay medium) MEM (with Earles salts, Gibco BRL 21090-022) containing 1% Nutridoma-SR (Boehringer BCL Cat. No. 1271091) and Penicillin + Streptomycin antibiotics at 50U/ml final concentration and Glutamine at 2mM final concentration.

The conditioned cells are now ready for addition of the various stimuli and or inhibitor compound.

A suitable experimental format comprises:

Cells + 500µl Assay medium
+ 25µl inhibitor or vehicle (zero value; 6.27% DMSO)
+ 25µl stimulus No.1 or Assay medium
+ 25µl stimulus No.2 or Assay medium

The following stimuli were used to induce the cells to release TGF-α:

Phorbol 12 - Myristate 13 - acetate (PMA, Sigma Cat. No. P8139).

Interferon-γ (IFN-γ,Boehringer Mannheim 100,000U/ml. Cat. No. 1040 596).

Interleukin-1ß (IL-1ß,Boehringer Mannheim 100,000U/ml. Cat. No. 1457 756).

LPS (lipopolysaccharide).

PMA was used at a final concentration of 5ng/ml.

IFN-γ was used at a final concentration of 100U/ml.

IL-1ß was used at a final concentration of 10U/ml.

The broad spectrum matrix metalloproteinase inhibitor compound was dissolved in 30% dimethyl sulphoxide (DMSO) as a 11mM stock solution and tested at 100µM (final DMSO concentration in assay = 0.27%).

After 5 hours or 24 hours at 37°C, 5%$CO_2$, samples of the conditioned media (225µl) were removed into a phosphate buffer saline protease inhibitor cocktail (Leupeptin (0.6µg/ml), Pepstatin (0.4µg/ml), Bestatin (5µg/ml), PMSF(phenyl-methyl-sulfonyl fluoride)(10µg/ml) and Phosphoramidon (10µg/ml), Sigma. concentrations given are final) and centrifuged at 4°C for 5 minutes at 300g. Aliquots of the supernatant were then assayed for TGF-α using a commercially available sandwich enzyme immunoassay (Oncogene Science Inc.).

Of the nineteen different human cell lines tested for their ability to produce TGF-α, either constitutively or following stimulation, only five released TGF-α sufficiently for assay purposes. Only HS294T and HT1080 released TGF-α constitutively.

The 2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid (compound 1) induced TGF-α inhibition obtained with these five cell lines is depicted in table 1.

TABLE 1.

CELL LINES EXHIBITING TGF-α PROCESSING AFTER 5 OR 24 HOURS CONDITIONING +/- 100μM compound 1

| CELL LINE | STIMULUS | TGFα pg/ml | | | |
|---|---|---|---|---|---|
| | | 5hr | | 24hr | |
| | | - | + comp. 1 | - | + comp.1 |
| HS294T | C | 130±4 | - | 335± 15 | - |
| | LPS | 138±7 | - | 266±4 | - |
| | PMA | 104±2 | - | 237 ±36 | - |
| | IFN-γ/IL-1β | 124 ± 12 | - | 177 ± 16 | - |
| OVCAR | C | - | - | - | - |
| | LPS | - | - | - | - |
| | PMA | 136±31 | - | 163±31 | - |
| | IFN-γ/IL-1β | - | - | - | - |
| HT1080 | C | 163±15 | 14±3 | 113±8 | 18±11 |
| | LPS | 154±29 | 12±3 | 88±16 | 30±5 |
| | PMA | 181±6 | 18±12 | 102±7 | 36±17 |
| | IFN-γ/IL-1β | 130±16 | - | 89±16 | 18±9 |
| A549 | C | - | - | - | - |
| | LPS | - | - | - | - |
| | PMA | - | - | 46±5 | - |
| | IFN-γ/IL-1β | - | - | - | - |
| Hela | C | - | - | - | - |
| | LPS | - | - | - | - |
| | PMA | 67±8 | - | 100±2 | 47±9 |
| | IFN-γ/IL-1β | - | - | - | - |

Background = (-) <10 pg/ml C = control, unstimulated comp. 1 = compound 1.

**Example 2**

Testing of various broad spectrum and specific matrix metalloproteinase inhibitors at inhibiting TGF-α from HS294T cells.

Three broad spectrum and two selective MMP inhibitor compounds were tested without stimulation of the cells, according to the method of example 1, with the modification that the test compound was used in the range of 0.4 - 100 μM. The test cells were HS294T melanoma cells which were incubated with the test compound for 24 hours. The concentration of TGF-α (pg/ml) was plotted versus inhibitor concentration and the $IC_{50}$ value (50% inhibitory concentration) calculated (see table 2).

Test compound

The following broad spectrum MMPI compounds were used:

Compound 1

2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid

Compound 2

3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-prop-2-enyl-hexanohydroxamic acid

Compound 3

3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-hydroxy-5-methyl-hexanohydroxamic acid

The following compounds used are enzyme selective MMPI compounds:

Compound 4

5-methyl-3R-(4-morpholinocarbonyl)-2S-phthalimidomethyl-hexanohydroxamic acid

Compound 5

3R-(1S-methylcarbamoyl-2-phenyl-ethylcarbamoyl)-6-phenylhexanoic acid

Table 2.

| Test compound | $IC_{50}$(nM) TGF-α | MMP Selectivity |
|---|---|---|
| 1 | 1500 | Broad Spectrum |
| 2 | 2500 | Broad Spectrum |
| 3 | 6000 | Broad Spectrum |
| 4 | >100,000 | COLLAGENASE |
| 5 | >100,000 | GELATINASE |

Only the broad spectrum matrix metalloproteinase inhibitor compounds are active at inhibiting TGF-α processing and release, possessing $IC_{50}$ values of less than or equal to 15μM.

**Example 3**

Testing of various broad spectrum matrix metalloproteinase inhibitor compounds in inhibiting TGF-α from HT1080 (human fibrosarcoma) cells.

Monolayers of HT1080 (human fibrosarcoma) cells (1.5 x $10^6$ cells/well of a 6-well plate) were incubated at 37°C overnight in DMEM + 10% foetal calf serum, supplemented with non-essential amino acids (Gibco-BRL). TGF-α release was stimulated with PMA at a final concentration of 1ng/ml in the presence of the compound to be tested at concentrations ranging from 200μM to 0.02μM. After 8 hours at 37°C, the culture supernatants were assayed for TGF-α using a commercially available sandwich enzyme immunoassay (Oncogene Science Inc.). The concentration of TGF-α (pg/ml) was plotted versus inhibitor concentration and

the $IC_{50}$ value calculated (see table 3).

Test compound

The following broad spectrum MMPI compounds were used:

Compound 3

3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-hydroxy-5-methyl-hexanohydroxamic acid

Compound 6

3R-(1S-methylcarbamoyl-2-phenylethylcarbamoyl)-5-methyl-2S-(thien-2-ylsulphanylmethyl)-hexanohydroxamic acid

Compound 7

R-(3-tert-butoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-(4-hydroxy-phenylsulphanylmethyl)-5-methyl-hexanohydroxamic acid

Compound 8

3R-(2,2-dimethyl-1S-(thiazol-2-ylcarbamoyl)-propylcarbamoyl)-5-methyl-2S-prop-2-enyl-hexanohydroxamic acid

Table 3.

| Test compound | Average $IC_{50}$ (nM) |
|---|---|
| 3 | 9700 n=10 |
| 6 | 1400 n=8 |
| 7 | 2800 n=8 |
| 8 | 900 n=9 |

Claims.

1. The use of an exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore, in the preparation of an agent for the treatment of conditions or diseases mediated or primarily mediated by transforming growth factor alpha (TGF-α).

2. A method of management (by which is meant treatment or prophylaxis) of diseases or conditions mediated or primarily mediated by TGF-α in mammals, particularly in humans, which method comprises administration to the mammal an effective dose of an exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore.

3. An exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore, for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated or primarily mediated by TGF-α.

4. A use as claimed in claim 1, a method as claimed in claim 2 or a compound for use as claimed in claim 3, wherein the exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore is selected from the group consisting of:
3R-(1S-methylcarbamoyl-2-phenylethylcarbamoyl)-5-methyl-2S-(thien-2-ylsulphanylmethyl)-hexanohydroxamic acid, 3R-(3-tert-butoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-(4-hydroxy-phenylsulphanylmethyl)-5-methyl-hexanohydroxamic acid, 3R-(2,2-dimethyl-1S-(thiazol-2-ylcarbamoyl)-propylcarbamoyl)-5-methyl-2S-prop-2-enylhexanohydroxamic acid, 2S-(4-hydroxy-phenylsulphanylmethyl)-3R-(3-methoxycarbonyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-hexanohydroxamic acid,
3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-5-methyl-2S-pr
op-2-enyl-hexanohydroxamic acid or 3R-(2,2-dimethyl-1S-methylcarbamoyl-propylcarbamoyl)-2S-hydroxy-5-methyl-hexanohydroxamic acid.

5. A use as claimed in claim 1 or claim 4, a method as claimed in claim 2 or claim 4 or a compound for use as claimed in claim 3 or claim 4, wherein the disease or condition referred to is psoriasis, scleroderma, hemangioma, diabetic retinopathy, neovascular glaucoma, retrolental fibroplasia, atherosclerosis, arteriovenous malformations, vascular adhesions, arthritis, wound healing, ultraviolet-induced skin hyperplasia, fibrotic disorders, systemic sclerosis, systemic lupus erythematosus, vasculaities, vasculitides, hypercalcemia of malignancy, ovarian carcinoma, breast cancer, liver cancer, squamous carcinoma, ovarian carcinoma, keratoacanthoma, pancreatic carcinoma, colon carcinoma or erythroleukaemia.

6. A pharmaceutical or veterinary composition adapted for inhibition of TGF-$\alpha$ release which composition comprises at least one exogenous broad spectrum matrix metalloproteinase inhibitor compound as defined hereinbefore, in admixture with at least one pharmaceutically or veterinary acceptable carrier.

# INTERNATIONAL SEARCH REPORT

Interr ial Application No
PCT/GB 96/00280

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/16 A61K31/165 A61K31/38 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 182, August 1995, pages 449-457, XP000574663 CONWAY, J.G. ET AL: "Inhibition of cartilage and bone destruction in adjuvant arthritis in the rat by a matrix metalloproteinase inhibitor" see the whole document --- | 1-3,5,6 |
| X | WO,A,94 10990 (BRITISH BIOTECHNOLOGY LIMITED) 26 May 1994 see the whole document especially page 17, compound 3 --- | 1-6 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 July 1996 | 19. 07. 96 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax ( + 31-70) 340-3016 | Mair, J |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/GB 96/00280

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | CANCER RESEARCH,<br>vol. 94, no. 6, December 1994,<br>pages 2177-2182, XP000574655<br>GIJBELS, K. ET AL: "Reversal of experimental autoimmune encephalomyelitis with a hydroxymate inhibitor of matrix metalloproteinases"<br>see the whole document<br>--- | 1-3,5,6 |
| X | ANNALS OF THE NEW YORK ACADEMY OF SCIENCES,<br>vol. 732, 1994,<br>pages 411-13, XP000575439<br>DIMARTINO, M.J. ET AL: "Preclinical antiarthritic activity of matrix metalloproteinase inhibitors"<br>see the whole document<br>--- | 1-3,5,6 |
| X | WO,A,90 05719 (BRITISH BIO-TECHNOLOGY LIMITED) 31 May 1990<br>cited in the application<br>see page 1, line 7-29<br>see page 34<br>--- | 1-6 |
| X | WO,A,94 21625 (BRITISH BIO-TECHNOLOGY LIMITED) 29 September 1994<br>see page 4, line 10-19<br>see page 27<br>--- | 1-6 |
| X | WO,A,93 20047 (BRITISH BIO-TECHNOLOGY LIMITED) 14 October 1993<br>cited in the application<br>see the whole document<br>especially page 10, line 8-12<br>----- | 1-6 |

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# INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/GB96/00280

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-6
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Annex

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/210**

The expression "an exogenous broad spectrum matrix metalloproteinase inhibitor compound" is not a clear and limited description of a chemical compound. The structural formula (I) on page 6 of the description contains substituents R1-R5 which are not defined. Because of this lack of adequate definition of the compounds intended to be used in the application a comprehensive search would involve a major part of the chemistry related IPC documentation. Such a search is not economically feasible. The search has therefore had to be restricted to those compounds specifically claimed and the general concept of the application.

The definition of the therapeutic application as the management of "diseases mediated ...by transforming growth factor alpha" is not a proper definition of the intended therapeutic use, because it is not fully known which conditions do and do not fall under this definition. The search has therefore had to be restricted to the claimed therapeutic applications and the general concept of the application.

**Remark:** Although claim 2 is directed towards a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compounds.

# INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/GB 96/00280

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| WO-A-9005719 | 31-05-90 | AU-B- | 644064 | 02-12-93 |
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